THE PERSONALIZATION OF CLOPIDOGREL ANTIPLATELET THERAPY IN CORONARY ARTERY DISEASE PATIENTS USING PHARMACOMETABONOMICS, PHARMACOGENETICS AND PLATELETS FUNCTION TESTING

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2016

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By

ARWA MOHAMED AMIN MOSTAFA

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

UNIVERSITI SAINS MALAYSIA
September 2016

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

سَنُرِيهِمۡ ءَايَٰتِنَا فِي ٱلۡأٓفَاقِ وَفِيٓ أَنفُسِهِمۡ حَتَّىٰ يَتَبَيَّنَ لَهُمۡ أَنَّهُ ٱلۡحَقُّۗ أَوَ لَمۡ يَكۡفِ بِرَبِّكَ أَنَّهُۥ عَلَىٰ كُلِّ شَيۡءٖ شَهِيدٌ ٥٣

سورة فصلت، آية 53

﴿We will soon show them Our signs in the horizons and within themselves, until it will become manifest to them that indeed it is the truth. Is it not sufficient in regard to your Lord that He is a witness over all things?﴾

Quran, Surat 41 (Fossilat): verse 53

DEDICATION

To my beloved parents,

To my dear siblings Moaaz, Anas, Baraa, Hamza and Somaya,

for your tremendous support, love and duaa that you always give to me,

To the shipmasters of this study, Dr Baharudin Ibrahim, Prof. Yuen Kah Hay,

Dr. Dzul Azri M. Noor, Dr. Lim Sheau Chin and Dr. M. Ali Sheikh Abdul Kader

for your encouragement, guidance, mentoring and support,

I would like to dedicate this humble work to you

ACKNOWLEDGMENT

All praises and thanks are due to ALLAH the Almighty and the giver of bountiful blessings and great favours. I would have not been able to accomplish this study, without His guidance, safeguarding and protection. Peace and prayers be on the prophet Muhammad (PBUH), the mercy to all mankind and the greatest teacher, who stated: "*Whoever does not give thanks to the people does not give thanks to ALLAH*". Therefore, I would like to show special thanks to all those who supported me throughout my studies.

This research could not have been accomplished without the tremendous guidance and support of my supervising panel, by whom it was a great honour to be supervised, supported and mentored. My deep gratitude and sincere appreciation are due to my main supervisor, Dr. Baharudin Ibrahim for providing me with this valuable opportunity to attain knowledge, to learn and to develop. I am grateful for his mentoring, guidance and support throughout my studies. The knowledge and experience that I have gained from him are precious, and so I would like to first thank him for contributing to my development and growth as an independent researcher while inspiring my creativity.

Secondly, I would like to express my deep gratitude and sincere appreciation to my first co-supervisor, Professor Yuen Kah Hay, and his post-doctoral assistant, Dr. Lim Sheau Chin, for their tremendous support, advice and untiring assistance throughout the entire study. Most notably, I would like to acknowledge them for including me in Prof. Yuen's research team where I gained considerable knowledge and experience, and established wonderful, lifelong friendships.

Thirdly, I would like to express my sincere gratitude to my second co-supervisor, Dr. Dzul Azri M. Noor, for his guidance, advice and support. I would like to thank him for his extended contributions and efforts throughout the entire study.

Furthermore, I would like to express my sincere gratitude to my field supervisor, Dr. M. Ali Sheikh Abdul Kader, for his unconditional support and tremendous help throughout the data collection work in the Hospital Pulau Pinang (HPP). I am deeply grateful for his advice and assistance throughout the study. I would also like to extend my thankfulness to the director of the cardiology department, HPP, Dato Dr. Omar Ismail, for facilitating the data collection of the study. I am grateful to the sisters and staff nurses of the cardiology department who assisted me in recruiting and drawing blood samples from the patients. My deep thanks also goes to the patients who participated in this study.

My thanks are further extended to Dr. Nurzalina Abdul Karim Khan for allowing me to carry out the genetic work in the Nutritix lab. I would like to express my sincere thankfulness to Dr. Teh Chin Hoe from Bruker, Sdn Bhd Malaysia for his efforts in adjusting the NMR experiments and identifying the biomarkers.

Additionally, I would like to thank the lab managers and staff of the clinical pharmacy and pharmaceutical chemistry laboratories of our school for assisting me in all of the lab work of the study, in a precise and timely manner. My sincere thanks are extended to my lab colleagues and fellow friends from Dr. Baharudin's and Prof. Yuen's research teams for their suggestions and contributions to my research.

A special thanks is due to my family. Words are not sufficient to express my sincere gratitude to my dear parents who I feel overwhelmingly grateful for their

unconditional love, prayers, caring and financial support of my education, and for all of the sacrifices that they have made on my behalf. My gratefulness is also extended to my dear brother Hamza who was always been on my side while caring, encouraging and supporting me throughout my educational journey. In addition, would like to thank my dear siblings; Moaaz, Anas, Baraa and Somaya for their love and support throughout these years. Lastly, I would like to thank my Malaysian and international friends for supporting me through the challenging times.

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LIST OF ABBREVIATIONS

µ	micro
% Inh	Percentage of Inhibition
ABW	Adjusted Body Weight
ACS	Acute Coronary Syndrome
ADP	Adenosine Diphosphate
ADR	Adverse Drug Reaction
ACCF	American College of Cardiology Foundation
ACG	American College of Gastroenterologist
AHA	American Heart Association
ACE-I	Angiotensin Converting Enzyme inhibitors
ABCB1	ATP-binding cassette, sub-family B, member 1
APPT	Activated Partial Thromboplastin Time
AUROC	Area Under the ROC Curve
BASE	Baseline PRU value
B-BIOREFCODE	Bruker Biofluid Reference Compound Database
BMRB	Biological Magnetic Resonance Data Bank
BMI	Body Mass Index
CES_1	Carboxyl $Esterase_1$
CCB	Calcium Channel Blockers
CVDs	Cardio-Vascular Diseases
CSF	Cerebrospinal Fluids
CD226	Cluster Differentiation
CAD	Coronary Artery Disease
CHD	Coronary Heart Disease
CKD	Chronic Kidney Syndrome
COX1	Cyclooxygenase 1
Cr Cl	Creatinine Clearance
CYP_{450}	Cytochrome P450
DAPT	Dual Antiplatelet Therapy
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid

EM	Extensive Metabolizers
GI	Gastro-intestinal
Hb	Haemoglobin
Hct	Haematocrit
HDL	High-density-lipoprotein
HF	Heart Failure
^{1}H-NMR	Proton nuclear magnetic resonance
HTPR	High on Treatment Platelet Reactivity
HRPR	High Residual Platelets Reactivity
HMDB	Human Metabolome Database
HTN	Hypertension
IHDs	Ischemic Heart Diseases
INR	International Normalized Ratio
IM	Intermediate Metabolizers
IAP	Interventional Angiographic Procedure
L	Litre
LTA	Light Transmission Aggregometry
LoF	Loss of Function
MS	Mass Spectroscopy
mRNA	messenger Ribonucleic Acid
MEA	Multiple Electrode platelet Aggregometry
MDR_1	Multi Drug Resistance-1
mg	milligram
NCDs	Non-communicable Diseases
NMR	Nuclear Magnetic Resonance
NYHA	New York Heart Association
PLS	Partial Least Square
PLS-DA	Partial Least Square Discriminant Analysis
PK	Pharmacokinetics
PD	Pharmacodynamics
PFT	Platelets Function Testing
PFA-100	Platelets Function Analyzer
PW	Platelet Works

PON1	Paraoxonase-1
PCI	Percutaneous Coronary Intervention
PM	Poor Metabolizers
POC	Point of Care
PCs	Principle Components
PCA	Principle Component Analysis
PG E_1	Prostaglandin E_1
PAR4-AP	Protease Activated Receptor-4 Activating Peptide
PAR-1	Protease Activated Receptor-1
PRU	P2Y12Reaction Unit
PPIs	Proton Pump Inhibitors
PT	Prothrombin Time
RNA	Ribonucleic Acid
RBCs	Red Blood Cells
ST	Stent Thrombosis
Sr Cr	Serum Creatinine
SNP	Single Nucleotide Polymorphisms
Tx-A_2	Thromboxane A_2
TIA	Transient Ischemic Attack
TMAO	Trimethyl-amine-N-oxide
TMA	Trimethylamine
iso-TRAP	Thrombin Receptor Activating Peptide
UM	Ultra Metabolizer
VASP-P	Vasodilator-stimulated Phosphoprotein Phosphorylation assay
VN	VerifyNow®
WBCs	White Blood Cells

LIST OF APPENDICES

PERSONALISASI TERAPI ANTIPLATELET CLOPIDOGREL DALAM KALANGAN PESAKIT PENYAKIT ARTERI KORONARI MENGGUNAKAN FARMAKOMETABONOMIK, FARMAKOGENETIK DAN PENGUJIAN FUNGSI PLATELET

ABSTRAK

Clopidogrel ialah ubat antiplatelet yang penting. Sesetengah pesakit yang mengambil rawatan clopidogrel mengalami kereaktifan platelet terhadap rawatan yang tinggi (HTPR) yang boleh menjurus kepada dapatan yang teruk. Genotip daripada *CYP2C19* telah dicadangkan untuk meramalkan gerak balas clopidogrel. Bagaimanapun, hal ini mungkin kurang mencukupi untuk meramal gerak balas. Analisis farmakometabonomik menggunakan spektroskopi resonans magnet nuklear (1H-NMR) boleh membantu untuk mengenal pasti penanda biologi terbaru HTPR dalam plasma dan air kencing. Matlamat utama projer ini adalah untuk menilai penggunaan farmakometabonomik, farmakogenetik dan pengujian fungsi platelet bagi tujuan personalisasi terapi antiplatelet clopidogrel dalam kalangan pesakit arteri koronori (CAD). Objektif sekunder merangkumi penentuan kekerapan polimorfisme *CYP2C19 *2* dan **3* dan kekerapan HTPR clopidogrel. Sebagai tambahan , kajian ini mensasarkan untuk menilai kesan polimorfisme *CYP2C19* dan faktor-faktor bukan genetik lain terhadap gerak balas clopidogrel. Seramai 89 pesakit CAD yang dirancang untuk prosedur intervensi angiografik IAP telah diambil dan digenotip untuk *CYP2C19*2* dan **3*. Daripada mereka, 71 orang pesakit diberikan clopidogrel 600 mg. Sampel darah dan air kencing diambil daripada pesakit sebelum dos muatan dan enam (6) jam selepas dos muatan untuk analisis farmakometabonomik. Pesakit telah dinilai untuk pengujian fungsi platelet (PFT) menggunakan peralatan VerifyNow-P2Y12 6

jam selepas dos muatan diberikan. Analisis multivariat digunakan untuk mencari metabolik pembeda. Daripada 89 pesakit yang diambil, 38 (42.7%) dan 15 (16.9%) masing-masing ialah pembawa mutasi heterozigot *(*1 / *2)* dan homozigot *(*2 / *2)* alel *CYP2C19*2*. Enam orang pesakit ialah pembawa jenis heterozigot alel *CYP2C19*3 (*1 / *3)*. Daripada 71 PFT pesakit yang dinilai, 27 (38%) orang pesakit menderita akibat HTPR clopidogrel. Min nilai unit tindak balas P2Y12 (PRU) bagi mutasi *(*2 / *2)* homozigot *CYP2C19*2* (232.73, SD: 33.100) nyata sekali lebih tinggi daripada pesakit dengan jenis tabii *(*1 / *1)* (178.17, 68.224) dan heterozigot *(*1 / *2)* (181.20, SD: 56.246) ($p<0.05$). Walau bagaimanapun, perbezaan nilai-nilai min PRU kedua-dua jenis tabii *(*1 / *1)* dan heterozigot *(*1 / *2)* adalah tidak signifikan.Apabila dibandingkan dengan kawalan, min PRU lebih tinggi secara signifikan (213.83, SD: 60.92) pada pesakit yang mengambil CCB ($p<0.05$) tetapi tidak dalam DM jenis 2 atau CKD. Perokok mempunyai nilai PRU lebih rendah yang signifikan jika dibandingkan dengan bukan perokok dan perokok lampau (165.16, SD: 48.76) ($p<0.05$). BMI mempunyai korelasi positif sederhana yang signifikan dengan nilai PRU ($r= 0.312$, $P=0.008$) tetapi umur tidak berhubung kait secara signifikan dengan PRU. Satu perkaitan negatif antara haemoglobin (Hb) dan hematokrit (Hct), dengan kedua-dua PRU and BASE, ialah signifikan (r-PRU / nilai P= Hb -0.421 / 0.0001, Hct -0.343 / 0.004 & r-BASE / nilai P= Hb -0.477 / 0.0001, Hct -0.424 / 0.0001). Bagaimanapun, tiada kolerasi signifikan yang ditunjukkan antara %Inh dan Hb dan Hct. Analisis farmakometabonomik plasma pra dos mendedahkan bahawa dua penanda biologi, asid metilmalonik dan sn-glisero-3-fosfokolina, mempunyai kaitan dengan HTPR clopidogrel. Dalam plasma pasca dos, asid asetoasetik ialah penanda biologi HTPR clopidogrel. Dalam air kencing, N-fenilasetilglisina and L Prolin ialah penanda biologi pra dos. Dalam air kencing pasca dos, penanda biologi ialah kolina,

D glukosa, kreatinina, kreatin, trimetil-amina-N-oksid (TMAO), glisina, trimetil-amina, asid fenilasetik, betaina, urea, metanol, taurina dan asid hipurik. Penemuan kami menunjukkan bahawa HTPR clopidogrel mempunyai faktor yang pelbagai dengan faktor-faktor genetik dan bukan genetik menyumbang kepadanya. Walaupun polimorfisme *CYP2C19* menyumbang kepada HTPR clopidogrel, genotip sahaja tidak mencukupi untuk penyesuaian terapi. Farmakometabonomik bukan hanya menemui penanda biologi terbaru bagi HTPR clopidogrel tetapi juga mendedahkan keadaan yang berkaitan dengannya.

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ABSTRACT

Clopidogrel is a substantial antiplatelet drug. Some patients on clopidogrel treatment suffer from high on treatment platelets reactivity (HTPR) which may lead to poor outcome. Genotyping of *CYP2C19* had been recommended to predict clopidogrel response. However, this might be insufficient to predict the response. Pharmacometbonomics analysis using nuclear magnetic resonance (^{1}H-NMR) spectroscopy can help to identify novel biomarkers of HTPR in plasma and urine. The primary aim of this project was to evaluate the use of pharmacometabonomics, pharmacogenetics and platelets function testing for the personalization of clopidogrel anti-platelet therapy in coronary artery disease patients. Secondary objectives included determining the frequency of *CYP2C19 *2* and **3* polymorphisms and the frequency of clopidogrel HTPR. Moreover, this study aimed to evaluate the effect of *CYP2C19* polymorphisms and other non-genetic factors on clopidogrel response. A total of 89 CAD patients planned for interventional angiographic procedure (IAP) were recruited and genotyped for *CYP2C19*2* and **3*. Out of those, 71 patients were loaded with clopidogrel 600 mg. Blood and urine samples were collected from patients before loading and 6 hours after loading for the pharmacometabonomics analysis. Patients were assessed for platelets function testing (PFT) using the VerifyNow-P2Y12 kit at 6 hours after loading. Multivariate analysis was used to find the discriminating metabolites. Out of the 89 recruited patients, 38 (42.7%) and 15 (16.9%) were carriers

of heterozygous *(*1/*2)* and homozygous mutations *(*2/*2)* of the *CYP2C19*2* allele, respectively. Six patients were carriers of heterozygous *CYP2C19*3* allele *(*1/*3)*. Out of the 71 PFT assessed patients, 27 (38%) patients were suffering from clopidogrel HTPR. The mean P2Y12 reaction unit (PRU) value of the *CYP2C19*2* homozygous mutation (*2/*2) (232.73, SD: 33.100) was significantly higher than patients with the wild type (*1/*1) (178.17, 68.224) and the heterozygous *(*1/*2)* (181.20, SD: 56.246) ($p<0.05$). However, the mean PRU values of both wild type *(*1/*1)* and heterozygous *(*1/*2)* were not significantly different. When compared to their controls, the mean PRU was significantly higher (213.83, SD: 60.92) in patients on CCB ($p<0.05$) but not in type 2 DM or CKD. Smokers had significantly lower PRU value compared to non-smokers and past smokers (165.16, SD: 48.76) ($p<0.05$). The BMI had significant medium positive correlation with the PRU value ($r= 0.312$, $P=0.008$) but the age was not significantly correlated with the PRU. A negative correlation between haemoglobin (Hb) and Haematocrit (Hct), and both the PRU and BASE, was significant (*r*-PRU / *P* value= Hb -0.421/0.0001, Hct -0.343/0.004 & *r*-BASE / *P* value= Hb -0.477/0.0001, Hct -0.424/0.0001). However, no significant correlations between the %Inh and Hb and Hct were indicated. Pre-dose plasma pharmacometabonomics analysis revealed that two biomarkers, the methylmalonic acid and the sn-glycero-3-phosphocholine, were associated with clopidogrel HTPR. In post-dose plasma, acetoacetic acid was the biomarker of clopidogrel HTPR. In urine, the N-phenylacetylglycine and L-Proline were the pre-dose biomarkers. In post-dose urine, the biomarkers were choline, D-glucose, creatinine, creatine, trimethyl-amine-N-oxide (TMAO), glycine, trimethyl-amine, phenyl-acetic acid, betaine, urea, methanol, taurine and hippuric acid. Our findings indicated that clopidogrel HTPR is multifactorial with genetic and non-genetic factors contributing to it. Although

CYP2C19 polymorphism is contributing to clopidogrel HTPR, genotyping alone is insufficient to tailor therapy. Pharmacometabonomics did not only discovered novel biomarkers of clopidogrel HTPR but also revealed conditions associated with it.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1. Background

1.1 The global burden of cardiovascular diseases (CVDs)

Cardiovascular diseases (CVDs) are the diseases which affect the heart and the circulatory system of the body such as coronary artery disease (CAD), stroke and acute coronary syndrome (ACS). Based on the World Health Organization, in 2008 the CVDs were one of the fatal non-communicable diseases (NCDs) which included: CVDs, cancer, diabetes and chronic lung diseases (World Health Organization, 2008). The CVDs represented the highest cause of death among NCDs globally (World Health Organization, 2008). Similarly, the health fact sheet of the Ministry of Health of Malaysia indicated that CVDs were the top leading cause of deaths in hospitals (World Health Organization, 2012; Ministry of Health, 2013).

1.2 Coronary artery disease (CAD)

Coronary artery disease is an atherosclerotic cardiac disease which affects the coronary arteries of the heart (American Heart Association, 2014). The pathophysiology of CAD includes the formation of plaque in one of the coronary arteries; in case of being enlarged, it may rupture and form the thrombus which causes narrowing of the artery and consequently ischemia (Libby and Theroux, 2005; HSF, 2009; American Heart Association, 2014). There are risk factors which can lead to CAD such as dyslipidemia, hypertension, the presence of pro-inflammatory cytokines

and diabetes (Libby and Theroux, 2005). The CAD, if not efficiently treated, may progress to coronary heart disease (CHD) and ischemic heart diseases (IHDs) (American Heart Association, 2014). This is because CAD patients have high platelets reactivity and tend to form monocyte-platelet aggregates (Furman et al., 1998). Based on the condition of the patient, the treatment of CAD may include several medications such as; ß-blockers, antiplatelet therapy, angiotensin converting enzyme inhibitors (ACE-I), calcium channel blockers (CCB), nitrates and anti-hyperlipidemics (Members et al., 2013).

1.3 Percutaneous coronary intervention (PCI)

Some of the CAD patients may require more invasive therapy such as; percutaneous coronary intervention (PCI) to open the occluded coronary artery (Torpy, Lynm, and Glass, 2004). The PCI procedure involves catheterization and balloon angioplasty (Torpy et al., 2004). A stent will be implanted in the occluded area to ensure enough blood flow through this area (Torpy et al., 2004). Patients who will be undergoing PCI procedure have to take a pre-procedure 600 mg loading dose of clopidogrel antiplatelet drug followed by post-procedure dual antiplatelet therapy (DAPT) of 81mg aspirin and 75 mg clopidogrel up to 12 months depending on the type of stent (Levine et al., 2011). This DAPT therapy is pivotal to prevent stent thrombosis and the recurrence of cardiac events (Levine et al., 2011).

1.4 Platelets activation and antiplatelet drugs

The platelets have substantial role in the development of atherosclerotic diseases by causing thrombus formation (Ruggeri, 1997; Libby and Theroux, 2005; Furie, 2009). The platelets rich thrombus usually consists of platelets aggregates and a net of fibrin (Ruggeri, 1997; Furie, 2009). The platelets become activated due to the interaction of several factors such as the adhesion of platelets to the ruptured plaque or exposed endothelium in the arteries, and the release of mediators such as adenosine diphosphate (ADP) thromboxane A_2 (Tx-A_2) and catecholamines (Zaman, Helft, Worthley, and Badimon, 2000; Furie, 2009). The activated platelets promotes further aggregation and activation of other platelets by the release of more ADP and Tx-A_2 (Dorsam and Kunapuli, 2004). Consequently, if the activation process continued, the platelets will aggregate with a mesh of fibrins and the thrombus will be formed (Furie, 2009). Thus, the mediators of platelets activation and their receptors had been considered the drug target for the inhibition of platelets in atherosclerotic diseases (Jakubowski, Winters, Naganuma, and Wallentin, 2007; Angiolillo and Luis Ferreiro, 2010; Nawarskas and Snowden, 2011). In figure 1.1, the platelets activation and the mechanism of action of some antiplatelet drugs are depicted.

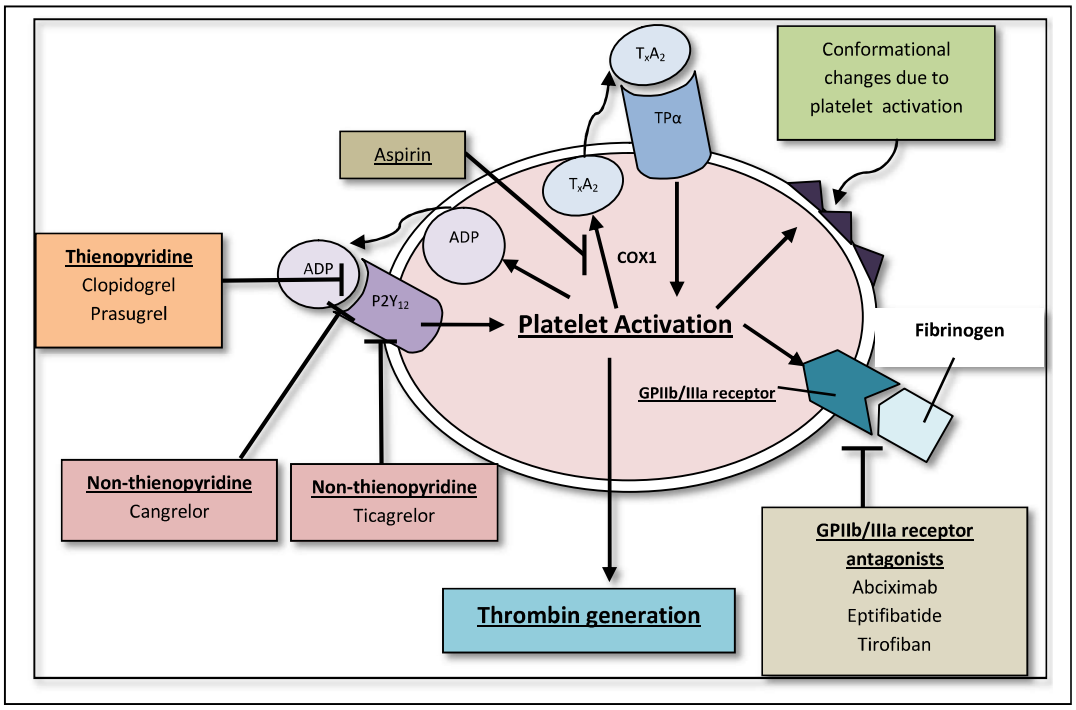

Figure 1.1: Platelets activation and the mechanism of action of some antiplatelet drugs

P2Y12 antagonists: inhibition of P2Y12 receptors, Aspirin: irreversible blockade of COX1 enzyme and GPIIb/IIIa antagonists: inhibition of GPIIb/IIIa receptors. ADP: adenosine diphosphate, COX1: cyclooxygenase-1, GPIIb/IIIa: glycoprotein IIb/IIIa, TPα: thromboxane α receptors, T_xA_2: thromboxane A_2.
Adapted and modified with permission from the publisher. Original source: (Nawarskas and Snowden, 2011).

1.4.1 P2Y12 receptor antagonists antiplatelet drugs

The adenosine diphosphate (ADP) activates platelets by the stimulation of two purinergic receptors, the P2Y1 and P2Y12 (Murugappa and Kunapuli, 2005; Angiolillo and Luis Ferreiro, 2010). The ADP stimulation of both receptors causes conformational changes of the platelets shape and instigates the formation of glycoprotein IIb/IIIa complex which leads to platelets aggregation (Angiolillo and Luis Ferreiro, 2010). However, since the P2Y12 receptor magnifies activation by other pathways, its effect dominates the effect of P2Y1 receptor in platelets activation (Dorsam and Kunapuli, 2004; Storey, 2006; Angiolillo and Luis Ferreiro, 2010;

Cattaneo, 2010). This renders the inhibition of P2Y12 receptor is substantial in reducing platelets aggregation (Storey, 2006).

The P2Y12 receptor antagonists are antiplatelet drugs which exerts its effect by the inhibition of the platelets' P2Y12 receptors (Angiolillo and Luis Ferreiro, 2010). Depending on their chemical structure, there are two main classes of P2Y12 antagonists; the thienopyridine and the non-thienopyridine (Jakubowski et al., 2007; Nawarskas and Snowden, 2011). Figure 1.2; shows the chemical structure of the thienopyridine and the non-thienopyridine P2Y12 antagonists.

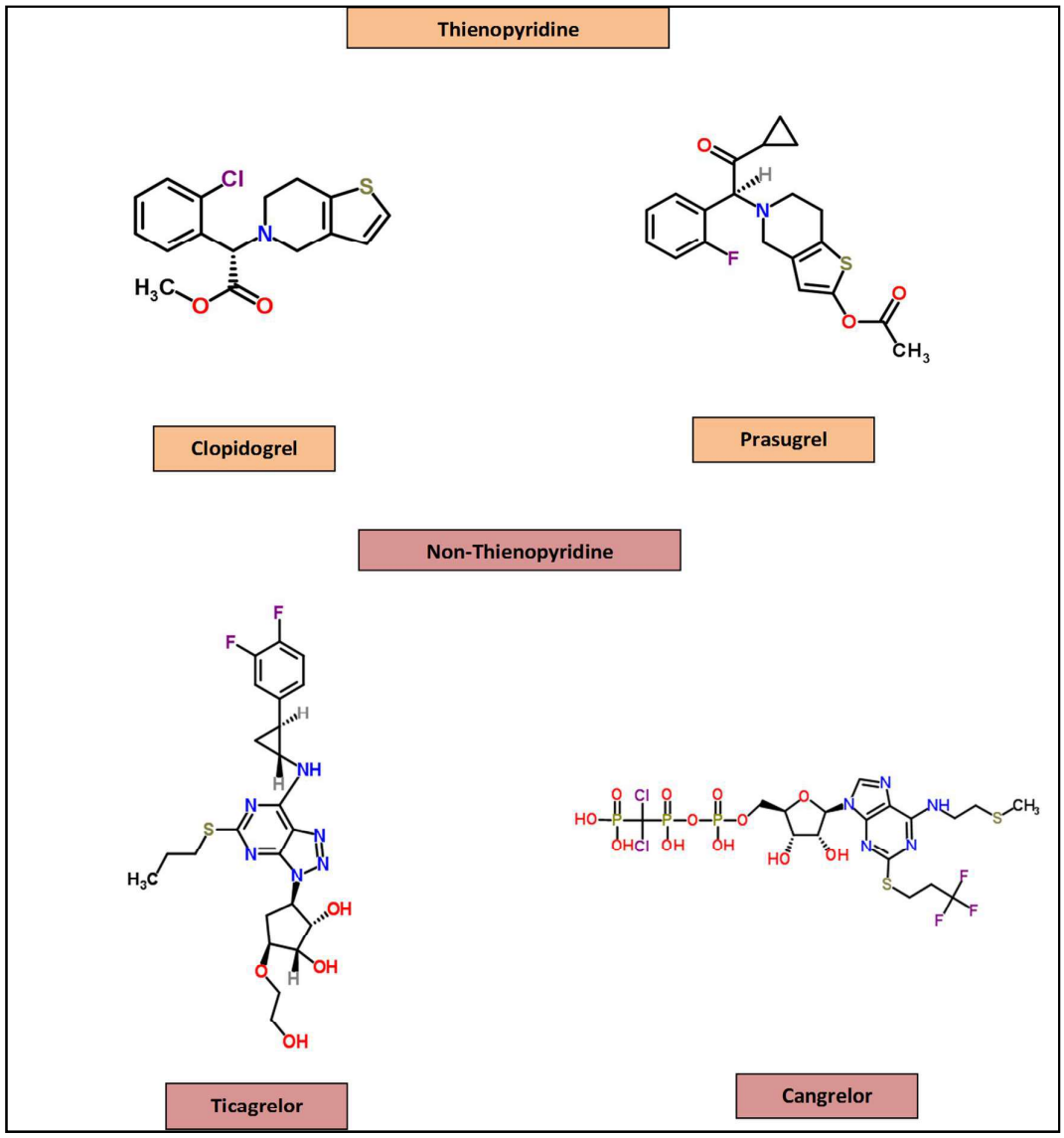

Figure 1.2: The Chemical Structure of the thienopyridine and the non-thienopyridine, platelets P2Y12 antagonists
Thienopyridine: Clopidogrel (Royal Society of Chemistry, 2014b) and Prasugrel (Royal Society of Chemistry, 2014c), Non-thienopyridine: Ticagrelor (Royal Society of Chemistry, 2014d) and Cangrelor (Royal Society of Chemistry, 2014a)

1.4.1.1 Thienopyridine P2Y12 receptor antagonists

The ticlopidine is the first generation thienopyridine antiplatelet drug. It was developed three decades ago (Saltiel and Ward, 1987). It is a prodrug which should be activated by the cytochrome P450 (CYP_{450}) enzymes to its active metabolite (Gent et

al., 1989; Cattaneo et al., 1991; Schrör, 1993). This active metabolite irreversibly inhibits the P2Y12 receptor (Quinn and Fitzgerald, 1999). Although several large studies indicated the beneficial effect of dual antiplatelet therapy of aspirin and ticlopidine in PCI patients (Schömig et al., 1996; Bertrand et al., 1998; Urban et al., 1998), the use of ticlopidine is hampered by its side effects (Maseneni, Donzelli, Taegtmeyer, Brecht, and Krähenbühl, 2012). The neutropenia and the thrombocytopenia were the most serious side effects of ticlopidine that sometimes lead to fatal outcome in PCI patients (Fukushima et al., 2007; Maseneni et al., 2012; Maseneni, Donzelli, Brecht, and Krahenbuhl, 2013). These side effects, in addition to other side effects such as liver dysfunction and skin rash (Fukushima et al., 2007), had reduced its use towards the second generation thienopyridine (clopidogrel) (Angiolillo and Luis Ferreiro, 2010; Maseneni et al., 2012).

Clopidogrel is the second generation thienopyridine. It was approved in 1997 after the Clopidogrel versus Aspirin in Patients at Risk Ischemic Events (CAPRIE) trial and since then it was available in the pharmaceutical market (Ringleb, Bhatt, Hirsch, Topol, and Hacke, 2004). The trial indicated that clopidogrel is more effective than aspirin in reducing the combined risk of ischemic stroke, myocardial infarction, or vascular death. Similar to ticlopidine, clopidogrel is a prodrug which needs enzymatic bioactivation by the cytochrome P450 (CYP) to its active metabolite which irreversibly inhibits the P2Y12 receptor of the platelets (Mega et al., 2009; Cattaneo, 2010). However, the bioactivation of clopidogrel is affected by inter-individual variability (Oprea and Popescu, 2013). In contrary to ticlopidine, clopidogrel has less neutropenic and thrombocytopenic side effects (Cattaneo, 2010; Maseneni et al., 2012). Therefore, it is well tolerated by patients (Maseneni et al., 2012) and to date,

clopidogrel remains the gold standard antiplatelet drug in the management of atherosclerotic diseases such as acute coronary syndrome (ACS), stroke and post PCI DAPT treatment (Angiolillo and Luis Ferreiro, 2010).

Prasugrel is the third generation thienopyridine (Gurbel and Tantry, 2008). Similar to the first and the second generations thienopyridine drugs, prasugrel is a prodrug which its active metabolite irreversibly inhibits the P2Y12 receptor (Niitsu, Jakubowski, Sugidachi, and Asai, 2005; Jakubowski et al., 2007). However, its activation is faster than clopidogrel and is not affected by inter-individual variability in activation (Jakubowski et al., 2007). In terms of platelets inhibition, it has better inhibition than clopidogrel (Wiviott et al., 2007). In the Trial to Assess Improvement in the Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction (TRITON-TIMI 38), prasugrel showed to be more efficient in reducing rates of ischemic events in ACS patients than clopidogrel (Wiviott et al., 2007). However, superiority of prasugrel against clopidogrel comes with a higher risk of bleeding, particularly in patients age more than 75 years old, weighing less than 60 kilograms (KG) and those who have history of stroke or transient ischemic attack (TIA) (Wiviott et al., 2007).

1.4.1.2 Non-thienopyridine P2Y12 receptor antagonists

Ticagrelor is a new oral non-thienopyridine antiplatelet drug which belongs to the cyclopentyl-triazolo-pyrimidines chemical class (Cattaneo, 2010). Unlike thienopyridine P2Y12 antagonists, ticagrelor acts by the non-competitive and selective block of the P2Y12 receptor (Nawarskas and Snowden, 2011). As ticagrelor binds to

a specific site on the P2Y12 receptor, other than the ADP site, its inhibition is reversible and the platelets can rejoin an activation process again (Nawarskas and Snowden, 2011). Ticagrelor is not a prodrug and has a rapid onset of action (Nawarskas and Clark, 2011; Nawarskas and Snowden, 2011). In the platelet Inhibition and Patient Outcomes (PLATO) trial ticagrelor was compared to clopidogrel in ACS patients and those who are undergoing PCI (all patients were on aspirin) (Wallentin et al., 2009). The results revealed that ticagrelor was preponderance in reducing the primary endpoint which was a composite of death from vascular causes, myocardial infarction, or stroke (Wallentin et al., 2009). However, it is worthy to note, the data of the PLATO trial has been challenged and it raised concerns and controversy (Coats, Nijjer, and Francis; Dinicolantonio and Biondi-Zoccai, 2013; DiNicolantonio and Serebruany, 2013; DiNicolantonio and Tomek, 2013; Steiner, Wu, and Ren, 2013). Moreover, further studies on the efficacy of twice daily dosing compared to clopidogrel are needed (Scott et al., 2013). The main side effects of ticagrelor are major and minor bleeding, dyspnoea and bradyarrhythmias (Wallentin et al., 2009; Nawarskas and Snowden, 2011; DiNicolantonio et al., 2013; Steiner et al., 2013).

Cangrelor is an intravenous (IV) novel non-thienopyridine anti-platelet drug which is an adenosine tri-phosphate ATP analogue (Cattaneo, 2010). It works by the selective direct, competitive, concentration dependent and reversible blockade of ADP binding to the P2Y12 receptor (Kubica et al., 2014). It is more potent than ticagrelor (Cattaneo, 2010). Due to its intravenous administration, cangrelor has a very rapid onset of action (Kubica et al., 2014). However, the Platelet Inhibition with Cangrelor in Patients Undergoing PCI (CHAMPION PCI) concluded that the difference in

reducing the composite endpoint (death from any cause, myocardial infarction, ischemia driven revascularization, stent thrombosis, stroke, and Q-wave myocardial infarction) at 48 hours, between clopidogrel oral loading dose of 600 mg, given 30 minutes before PCI and cangrelor IV given 30 minutes before PCI and continued for two hours, is not significant (Harrington et al., 2009). Therefore, further clinical trials are required to confirm cangrelor's efficacy and safety.

1.5 Clopidogrel

Although clopidogrel was approved since 1997, its role in the dual antiplatelet therapy of ACS was only substantiated in 2002, after the results of the Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) trial (Yusuf, Bijsterveld, and Moons, 2001). The CURE trial had indicated the improved outcome of adding clopidogrel to aspirin antiplatelet therapy in ACS patients without ST-segment elevation. As aspirin works by the irreversible blockade of COX-1 enzyme, the improved outcome is due to the additional platelet inhibition of clopidogrel by blocking the P2Y12 receptor as well (Yusuf et al., 2001). The pharmacological properties and the sufficient safety margin; in terms of bleeding and side effects, had established clopidogrel as the essential antiplatelet therapy paired with aspirin (Jakubowski et al., 2007; Angiolillo and Luis Ferreiro, 2010). To date, clopidogrel stays on the throne of P2Y12 antagonists in the DAPT of PCI patients to prevent stent thrombosis and recurrence of ischemic events. Although clopidogrel administration is crucial for PCI patients, its use is hindered by an inter-individual response variability. Indeed, some patients have high residual platelet reactivity (HRPR) on treatment of clopidogrel (Garabedian and Alam, 2013). This variability is a great challenge for the

optimum DAPT because it might lead to stent thrombosis, poor therapeutic outcome, recurrence of ischemic events and consequently death (Buonamici et al., 2007; Mega et al., 2009).

1.5.1 Pharmacology of clopidogrel

Clopidogrel is an inactive prodrug which needs two steps of bioactivation to its thiol active metabolite (CTM or R130964) by the hepatic CYP_{450} enzymes (Mega et al., 2009; Oprea and Popescu, 2013; Karaźniewicz-Łada et al., 2014). This active metabolite works by the irreversible inhibition of the P2Y12 receptor of the platelet by the formation of covalent disulfide bond with the extracellular cysteine residues on the receptor (Cattaneo, 2010; Ferri, Corsini, and Bellosta, 2013; Kubica et al., 2014). As the inhibition is irreversible, it will inactivate the receptor for any future stimulation by ADP for the life span of the platelet (7-10 days) (Kubica et al., 2014; Shameem, Hamid, Randhawa, Spaccavento, and Garatt, 2014). Consequent to the inhibition of the P2Y12 receptor, further GPIIb/IIIa receptor activation will not be achieved (Ferri et al., 2013; Shameem et al., 2014). This will prevent the formation of the conformational shape changes of the platelet and the formation of the GPIIb/IIIa-fibrinogen complex (Angiolillo and Luis Ferreiro, 2010; Nawarskas and Snowden, 2011). Subsequently, platelets aggregation will not be stabilized (Shameem et al., 2014).

1.5.2 Pharmacokinetics of clopidogrel

Clopidogrel has an oral bioavailability of 50% and the maximum peak concentration will be observed within 1 to 2 hours after the administration of the loading dose (600 mg) (Taubert et al., 2004; Oprea and Popescu, 2013). The half-life of clopidogrel is from 7 to 8 hours (Schrör, 1998). A daily dose of clopidogrel 75 mg will achieve the maximum plateau of platelet inhibition after 4 to 5 days of administration (Cattaneo, 2010). Almost 50% of clopidogrel dose is excreted in the urine and 46% in the faeces (Shepard, 2000). Of the oral dose, approximately 85% is hydrolyzed by esterases into inactive metabolite while the remaining 15% will be activated by the hepatic CYP_{450} enzymes to the active metabolite through the two steps of bioactivation (Shameem et al., 2014). The first step will form the immediate precursor which is the 2-oxo-clopidogrel and the second step will form the active metabolite (Kazui et al., 2010). The hepatic CYP_{450} enzymes, which are involved in the bioactivation process of clopidogrel, are the CYP1A2, CYP2B6 and CYP2C19 in the first step and the CYP2B6, CYP2C9, CYP3A4/5 and CYP2C19 for the second step (Kazui et al., 2010; Scott et al., 2013; Priyadharsini et al., 2014). The CYP2C19 enzyme plays the vital role in the two steps of clopidogrel bioactivation by participating with 44.9% in the first step and 20.6 % in the second step (Holmes et al., 2010; Kazui et al., 2010). The CYP3A4 has an essential role in the second step by participating with 39.8 % (Kazui et al., 2010). Clopidogrel and its metabolites have high protein binding properties which can be more than 98% (Ganesan, Williams, Maslen, and Cherala, 2013). The two steps of clopidogrel bioactivation and the contribution of the CYP_{450} enzymes to this bioactivation are illustrated in figure 1.3.

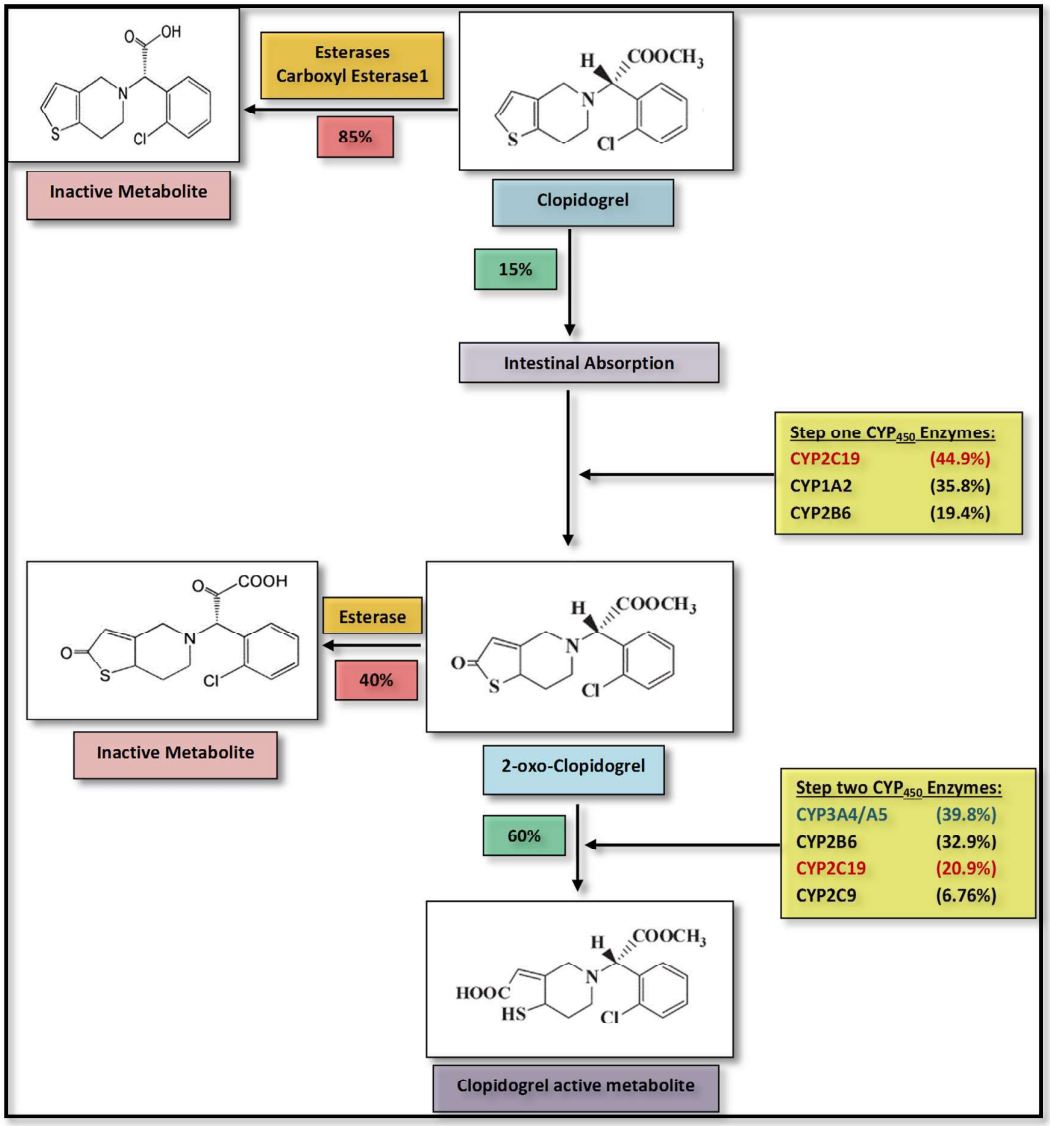

Figure 1.3 The clopidogrel two steps bioactivation and the contribution of CYP_{450} enzymes
(Adapted and modified with permission from the publisher. Original source: (Ma and Lu, 2011)

1.5.3 Side effects of clopidogrel

Compared to other P2Y12 antagonists, clopidogrel has minimum side effects (Wiviott et al., 2007; Cattaneo, 2010; Maseneni et al., 2012). The main side effects of clopidogrel are bleeding, gastro-intestinal disorders and rash (Cattaneo, 2010; Maseneni et al., 2012; Maseneni et al., 2013). Other side effects, albeit rare, include

hepatotoxicity and thrombotic thrombocytopenic purpura (Bennett et al., 2000; Ng, Goldberg, and Tafreshi, 2006; Maseneni et al., 2012; Maseneni et al., 2013). The clopidogrel may cause gastro-intestinal (GI) bleeding in patients having pre-clopidogrel treatment and GI ulcers (Ng et al., 2003), especially if it was used concomitantly with other antiplatelet drugs such as aspirin (Hallas et al., 2006; Abraham et al., 2010). This necessitates prophylactic treatment with proton pump inhibitors (PPIs) or histamine H_2 receptors antagonists (H_2RAs) with DAPT (Abraham et al., 2010). The American College of Cardiology Foundation (ACCF) / American College of Gastroenterologist (ACG) / American Heart Association (AHA) released a focused update in 2010 of the 2008 clinical expert consensus document, where they advocated the use of PPIs in patients on DAPT to prevent GI bleeding (Abraham et al., 2010).

1.5.4 Drug-drug interactions of clopidogrel

As clopidogrel is bioactivated by the CYP_{450} enzymes, it is affected by drug-drug interactions, particularly the drugs which interfere with the CYP_{450} system (Bates, Lau, and Angiolillo, 2011). The PPIs are metabolized by the CYP_{450} enzymes, therefore, they might interact with clopidogrel (Abraham et al., 2010). In fact, the PPIs inhibit the CYP2C19 enzyme which might reduce the bioactivation of clopidogrel to its active metabolite (Siller-Matula et al., 2009; Abraham et al., 2010; Bates et al., 2011). Accordingly, this could decrease the antiplatelet effect of clopidogrel and hence might increase the cardiac events (Bates et al., 2011). The PPIs do not interact to the same extent with clopidogrel (Siller-Matula et al., 2009). In other words, some studies concluded that omeprazole was associated with the reduced antiplatelet effect of

clopidogrel, while esomeprazole and pantoprazole were not (Siller-Matula et al., 2009; Bates et al., 2011). Nevertheless, the data on the extent of PPIs interaction with clopidogrel is still controversial which suggests further investigations (Abraham et al., 2010). The ACCF / ACG) / AHA 2010 update of the 2008 clinical expert consensus document suggested to separate the timing between the administration of the two drugs to limit the interaction between them (Abraham et al., 2010).

The lipophilic statins (antihyperlipidemic drugs) such as atorvastatin and simvastatin were reported to inhibit the antiplatelet effect of clopidogrel (Lau et al., 2003; Neubauer, Günesdogan, Hanefeld, Spiecker, and Mügge, 2003; Schmidt et al., 2012). These statins interfere with the CYP3A4 enzyme (Lau et al., 2003; Bates et al., 2011). Thus, it can mitigate the antiplatelet effect of clopidogrel (Lau et al., 2003). However, the preventive effect of statins by reducing lipids' level can halt cardiac events which might happen consequent to the reduced antiplatelet effect of clopidogrel (Schmidt et al., 2012).

Nevertheless, of a controversy in the literature, some drugs such as CCBs, erythromycin, troleandomycin and ketoconazole were reported to reduce the antiplatelet effect of clopidogrel by the interference with the CYP3A4 enzyme (Farid et al., 2007; Bates et al., 2011; Good et al., 2012). Similar to statins, those drugs are inhibitors of the CYP3A4 (Bates et al., 2011). It is suggested that these drugs might reduce the level of the clopidogrel's active metabolite which can lead to reduced antiplatelet effect of clopidogrel (Bates et al., 2011; Good et al., 2012). However, the data from some studies did not support this suggestion (Olesen et al., 2011; Good et al., 2012). For instance, the Clopidogrel for the Reduction of Events During Observation (CREDO) trial indicated that the concomitance use of the CCBs do not

reduce the efficacy of clopidogrel and there was no evidence of interaction between them (Good et al., 2012).

Anti-coagulant drugs such as coumarin derivatives may also interfere with platelets inhibition by clopidogrel. Sibbing et al. (2010) indicated a significant reduction in the platelets' inhibitory effect of clopidogrel among patients concomitantly taking phenoprocoumon when compared to those who are not taking phenoprocoumon. This is assumed to be because of the interference with the capacities of the CYP3A4 and the CYP2C9 enzymes which are the main metabolizing enzymes of the phenoprocoumon (Sibbing, Dirk et al., 2010). Sulfonylureas oral hypoglycemic drugs may also reduce platelets inhibitory effect of clopidogrel (Harmsze et al., 2011). This could be due to the interference with CYP2C9 enzyme, as well (Bell, 2004; Harmsze et al., 2011).

As there are drugs that reduce or increase the level of clopidogrel's active metabolite by the interference with CYP_{450} enzymes, there are also drugs which can increase or decrease the level of clopidogrel's active metabolite by the inhibition or induction of the carboxyl $esterase_1$ (CES_1); the hydrolytic enzyme which hydrolyzes clopidogrel, the intermediate metabolite (2-oxo-clopidogrel) and the active metabolite to their inactive metabolites (Figure 1.3) (Zhu et al., 2013). This might lead to variable response of clopidogrel (Siller-Matula, Trenk, Krähenbühl, Michelson, and Delle-Karth, 2014). For instance, the phenobarbital, the dexamethasone and the polycyclic aromatic hydrocarbons are drugs which can interfere with the activity of CES_1 which might lead to variable levels of clopidogrel's active metabolite (Siller-Matula et al., 2014).

1.6 Clopidogrel variable response

Eventhough the essential antiplatelet role of clopidogrel is to prevent stent thrombosis (ST) post PCI, this role is not adequately achieved in every patient. In fact, some patients do not respond to clopidogrel efficiently. The inter individual response variability of clopidogrel was firstly reported by Jaremo et al in 2002 (Järemo, Lindahl, Fransson, and Richter, 2002). In this study, the researchers found that five out of the eighteen PCI patients included in the study had weak platelets inhibition in response to clopidogrel loading dose of 300mg (Järemo et al., 2002). Since it was first reported, the documentation of the clopidogrel hyporesponsiveness has largely grown and it became well established (Gurbel and Tantry, 2006; Angiolillo, Fernandez-Ortiz, et al., 2007; Mega et al., 2009; Shuldiner et al., 2009; Scott et al., 2013). The clopidogrel hyporesponsive patients have high residual platelet reactivity (HRPR) or high on treatment platelet reactivity (HTPR) (Bonello, Tantry, et al., 2010; Garabedian and Alam, 2013). The HTPR was found to be affecting 15 - 40 % of the patients (Gurbel and Tantry, 2006; Gurbel and Tantry, 2007; Oprea and Popescu, 2013).

1.6.1 The association between the clopidogrel variable response and the clinical outcomes

As clopidogrel had been proven to improve the clinical outcomes of patients after PCI (Mehta et al., 2001; Eisenstein et al., 2007), the reports on its variable response instigated the researchers to study the effect of this response variability on the clinical outcomes. Several studies sought to evaluate the association between clopidogrel hyporesponsiveness and the clinical outcomes. In 2004, Matetzky and colleagues indicated an association between clopidogrel resistance and the risk of

cardiac events' recurrence among 60 ACS patients undergoing PCI who had taken loading dose of 300mg followed by daily dose of 75 mg for three months (Matetzky et al., 2004). These results were substantiated by the findings of Geisler et al., in 2006 (Geisler et al., 2006). In this study, the clinical outcomes of 379 patients were followed-up for three months after the PCI (Geisler et al., 2006). It was found that the primary end point of myocardial infarction, stroke and death were significantly increased in patients who were hyporesponsive to clopidogrel (Geisler et al., 2006). In 2007, a meta-analysis which included 25 studies of 3688 patients, had concluded that nonresponsive clopidogrel patients had higher risk of getting poor cardiovascular outcomes after the PCI (Snoep et al., 2007). In the same study, it was found that using 600 mg dose of clopidogrel may reduce this risk (Snoep et al., 2007). Since that time, an overwhelming literature continued to establish the association between clopidogrel hyporesponsiveness and the clinical outcomes (Price et al., 2008; Marcucci et al., 2009; Mega, Simon, et al., 2010; Brar et al., 2011).

1.6.2 High on treatment platelet reactivity (HTPR)

There are several terms which describe the low response to clopidogrel. Based on the diligence of each author; the clopidogrel resistance, the clopidogrel hyporesponsiveness, the high on treatment platelet reactivity (HTPR) and high residual platelets reactivity (HRPR), are commonly used terms in the literature to refer to clopidogrel's low responsiveness. However, they are not exactly the same. Simply put, the responsiveness is the difference between two measurements of platelets reactivity pre and post administration of an antiplatelet drug, where post administration measurement should be after the achievement of the full antiplatelet effect (Samara, Bliden, Tantry, and Gurbel, 2005; Bonello, Tantry, et al., 2010). In order to indicate

the responsiveness state of a patient, a clinically proven laboratory assay which measures platelets function pre and post administration is used (Bonello, Tantry, et al., 2010). While the clopidogrel resistance is the absolute non responsiveness to clopidogrel effect (no or minimal difference between pre and post values), the hyporesponsiveness may describe patients who slightly respond but not fully responsive (Ferguson, Dokainish, and Lakkis, 2008; Bonello, Tantry, et al., 2010; Gasparyan, 2010). The definitions of the resistance and the hyporesponsiveness vary depending on the defined cut-off values of each platelets function assay (Ferguson et al., 2008; Gasparyan, 2010). The high on treatment platelet reactivity (HTPR) or high residual platelets reactivity (HRPR) could be defined as an increased platelets reactivity after the achievement of the full antiplatelet effect of the loading dose of an antiplatelet drug (Garabedian and Alam, 2013). The HTPR and HRPR are considered superior to the responsiveness in estimating the risk of ischemic events (Samara et al., 2005; Bonello, Tantry, et al., 2010). This is because they are reflective to the current state of platelets reactivity (while on treatment) regardless of the baseline (Samara et al., 2005; Bonello, Tantry, et al., 2010). Currently, it is well documented that the HTPR is associated with the risk of having cardiovascular events while being on antiplatelet treatment (Marcucci et al., 2009; Bonello, Tantry, et al., 2010; Brar et al., 2011; Garabedian and Alam, 2013).

1.6.3 Factors contributing to clopidogrel HTPR

Upon the emergence of the data on clopidogrel variable response, the researchers were encouraged to explore the mechanisms which cause this variability. Studies which investigated the mechanisms of the variable response of clopidogrel

found that this variable response is multifactorial which can be attributed to genetic and non-genetic factors (Shuldiner et al., 2009; Bouman et al., 2011; Su et al., 2012; Ferri et al., 2013; Lewis, Horenstein, et al., 2013; Scott et al., 2013). In figure 1.4, the genetic and non-genetic factors which may contribute to clopidogrel HTPR are demonstrated.

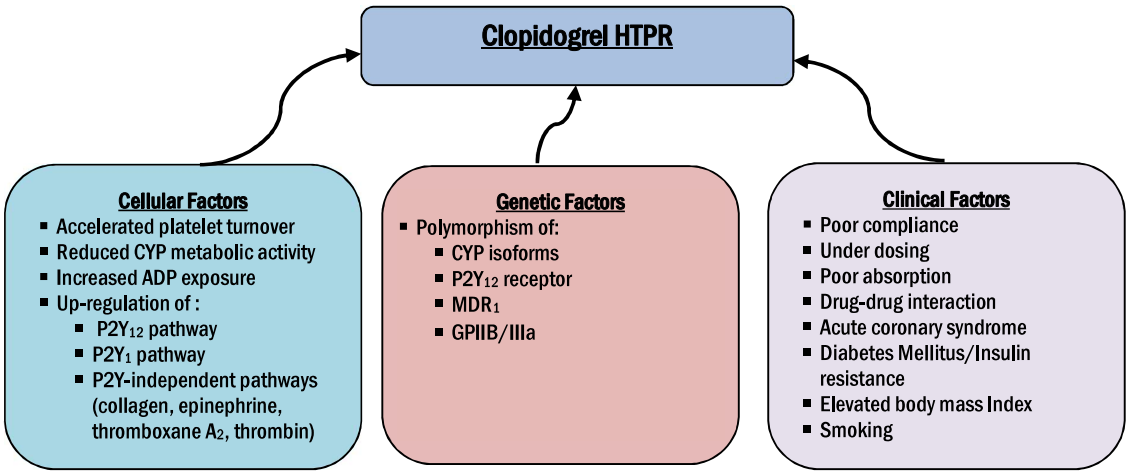

Figure 1.4: Genetic and non-genetic factors contributing to clopidogrel response ADP: adenosine diphosphate, CYP: cytochrome P450, GP: Glycoprotein, HTPR: high on treatment platelet reactivity, MRD1: Multidrug resistance transporter Adapted and modified with permission from the publisher. Original source: (Angiolillo and Luis Ferreiro, 2010).

1.6.3.1 Genetic factors contributing to HTPR

As clopidogrel undergoes intestinal absorption, bioactivation by the hepatic CYP_{450} enzymes and deactivation by esterases, this process might be affected by several genetic variabilities. This has been concluded in both genome-wide association studies (GWAS) and in particular gene association studies (Shuldiner et al., 2009; Mega, Close, et al., 2010; Yin and Miyata, 2011; Su et al., 2012; Lewis, Horenstein, et al., 2013). In fact, the variable response to clopidogrel is highly heritable (Shuldiner et al., 2009; Yin and Miyata, 2011; Cuisset, Morange, and Alessi, 2012). There are several identified genetic variabilities which contribute to the variable response of clopidogrel such as; the *CYP2C19* (Shuldiner et al., 2009), the ATP-binding cassette,

sub-family B, member 1 (*ABCB1*) (Su et al., 2012), the Paraoxonase-1 (*PON1*), the *CYP3A4/5* (Angiolillo et al., 2006; Suh et al., 2006) and the *CES1* (Lewis, Horenstein, et al., 2013). In addition, a genetic polymorphism of the *P2Y12* receptors had been reported to affect the response to clopidogrel (Staritz et al., 2009).

The CYP2C19 enzyme is involved in both the two bioactivation steps of clopidogrel as shown previously in figure 1.3. The *CYP2C19* gene has polymorphic alleles which causes the formation of enzyme with decreased or increased activity (Geisler, Schaeffeler, Gawaz, and Schwab, 2013). A functional genetic polymorphism of the *CYP2C19* was found to be the most contributing genetic variability to clopidogrel variable response (Mega et al., 2009; Shuldiner et al., 2009). The reduced activity polymorphism of the *CYP2C19* can cause reduced exposure to the active metabolite of clopidogrel which eventually will lead to the reduced inhibition to platelets reactivity (Geisler et al., 2013). Studies had proved that the *CYP2C19* genetic variant is a predictor of clopidogrel responsiveness and clinical outcomes (Mega et al., 2009; Shuldiner et al., 2009; Xie et al., 2013). The genetic variability of the *CYP2C19* will be discussed in the latter section of this chapter.

Both CYP3A4 and CYP3A5 enzymes are majorly involved in the second step of clopidogrel bioactivation as shown in figure 1.3. The literature on the effect of their genetic variants on clopidogrel response is controversial (Angiolillo and Luis Ferreiro, 2010). Some early studies indicated an association between, both, the *CYP3A4* and the *CYP3A5* genetic variants and the reduced response to clopidogrel (Angiolillo et al., 2006; Suh et al., 2006), whereas later studies failed to prove this association (Smith et al., 2006; Fontana, Hulot, De Moerloose, and Gaussem, 2007; Mega et al., 2009; Park, J. J. et al., 2013). Nonetheless, the *CYP3A5* - response association was asserted in

recent ethnicity study. Priyadharsini et al., (2014) conducted a study on Tamilian CAD patients in which *CYP3A5*3* polymorphism was found to be significantly contributing to clopidogrel resistance (Priyadharsini et al., 2014).

The *ABCB1* gene is the coding gene for the P-glycoprotein multi drug resistance-1 (MDR_1) intestinal transporter, a modulator of clopidogrel absorption (Angiolillo and Luis Ferreiro, 2010; Jaitner et al., 2012). A genetic polymorphism of the *ABCB1* cause reduced absorption of clopidogrel (Su et al., 2012). Simon et al., (2009) found that ACS patients who were treated with clopidogrel and were carriers of the two variants alleles of the *ABCB1* had higher rate of cardiovascular events at one year of follow-up (Simon et al., 2009). Similarly, Mega et al., (2010) indicated that the *ABCB1* polymorphism is significantly associated with the risk of deaths due to cardiovascular events or stroke among ACS patients treated with clopidogrel in TRITON-TIMI 38 trial (Mega, Close, et al., 2010). However, other studies failed to indicate the same results (Shuldiner et al., 2009; Jaitner et al., 2012).

The CES_1 enzyme is the main hydrolytic metabolizing enzyme of clopidogrel, its intermediate metabolite and its final bioactive metabolite, to carboxylic acid derivatives inactive metabolites (Figure 1.3) (Lewis, Horenstein, et al., 2013). As the majority of clopidogrel dose is being metabolized by CES_1 (Scott et al., 2013), a reduced function genetic polymorphism of this enzyme can lead to a great variable response to clopidogrel (Lewis, Horenstein, et al., 2013; Zhu et al., 2013). Zhu et al., (2013) found that the CES_1 inhibition and the *CES1* reduced function genetic polymorphisms which was discovered by their laboratory (Zhu et al., 2008), are associated with the reduced hydrolytic metabolism of clopidogrel and the increased concentrations of clopidogrel's active metabolite in an in vitro model (Zhu et al., 2013).

Concurrently, Lewis et al., (2013) studied the effect of this reduced function genetic variant of the CSE_1 on clopidogrel response among 566 healthy volunteers from the Amish Pharmacogenomics of Anti-Platelet Intervention (PAPI) study. The study revealed that the level of clopidogrel active metabolite is significantly higher among carriers of the reduced function allele (Lewis, Horenstein, et al., 2013). In addition, the platelets inhibition measured by an ADP-stimulated platelet aggregation was higher in those who were carriers of the reduced function allele. The findings were proved in 350 clopidogrel treated coronary heart disease patients (Lewis, Horenstein, et al., 2013).

The PON_1 is an esterase enzyme which is synthesized in the liver (Bouman et al., 2011). The *PON1*, full enzymatic activity, is associated with atheroprotective effect which can be due to its role in enhancing an increased level of the high-density-lipoprotein (HDL) (Bhattacharyya et al., 2008; Lewis and Shuldiner, 2012; Park, K. W. et al., 2013). Therefore, patients who were carriers of the lower activity genetic polymorphism of *PON_1* were found to be having an increased oxidative stress and higher risk of cardiovascular events (Bhattacharyya et al., 2008). A presumed role of *PON_1* in the metabolism of clopidogrel and its responsiveness stemmed controversy in the past few years (Lewis and Shuldiner, 2012). Bouman et al., (2011) used an in-vitro metabolomic profiling which indicated that the PON_1 is imperative enzyme in the hydrolytic cleavage of the intermediate metabolite of clopidogrel (2-oxo-clopidogrel) to the active metabolite of clopidogrel (Bouman et al., 2011). Accordingly, the genetic variant which leads to the formation of PON_1 enzyme with lower activity will cause lower level of clopidogrel's active metabolite (Bouman et al., 2011). Based on their findings, they conducted a case-cohort study on CAD patients

undergoing PCI and a further prospective replication study on another independent sample of 1982 patients with ACS (Bouman et al., 2011). Both the case-cohort and the prospective replication studies concluded that there was an association between the genetic variant of *PON1* and the pharmacokinetics, the pharmacodynamics and therapeutic outcome of clopidogrel (Bouman et al., 2011). These findings were challenged by other studies which indicated that this association is not significant (Sibbing et al., 2011; Ancrenaz et al., 2012; Chan et al., 2012; Chen et al., 2012; Gong et al., 2012; Kreutz et al., 2012; Lewis and Shuldiner, 2012; Paré et al., 2012; RENY, Combescure, Daali, and Fontana, 2012). The effect of *PON1* genetic variant on the therapeutic outcome was attributed to the association between this variant and the risk of developing cardiac events (Lewis and Shuldiner, 2012; Park, K. W. et al., 2013).

The P2Y12 receptor was also found to be affected by genetic polymorphism which might cause an increased in atherothrombosis and interfere with the response to clopidogrel (Fontana et al., 2003). This genetic polymorphism had shown to increase platelets activation and contribute to the decreased response to clopidogrel in CAD patients (Staritz et al., 2009). In fact, there are two haplotypes of the P2Y12 receptor gene (H1 and H2) where the H2 was found to cause this disequilibrium (Fontana et al., 2003; Staritz et al., 2009; Geisler et al., 2013). The data on the effect of the P2Y12 genetic variants on clopidogrel response is dialectical. Some studies supported an effect of the P2Y12 polymorphism on platelets activation and clopidogrel response (Fontana et al., 2003; Staritz et al., 2009; Zoheir et al., 2013), while others refuted this effect (von Beckerath et al., 2005; Bonello, Bonello-Palot, et al., 2010) or indicated a synergistic effect of it, if co-existed with other genetic variabilities such as *CYP2C19* and MDR1 (Shalia, Shah, Pawar, Divekar, and Payannavar, 2013; Tang et al., 2013).

Some studies found that having more than one genetic polymorphism can increase the chances of getting poor response to clopidogrel. For instance, a study concluded that PCI patients who may have both *ABCB1* and *CYP2C19* genetic variants may be vulnerable to the recurrence of cardiac events while they are on clopidogrel treatment (Mega, Close, et al., 2010). Similarly, studies indicated that coexistence of *CYP2C19* and *P2Y12* receptor genetic polymorphisms has higher effect on clopidogrel's responsiveness and the clinical outcome than single polymorphism (Shalia et al., 2013; Tang et al., 2013).

1.6.3.2 Non-genetic factors contributing to clopidogrel HTPR

In addition to genetic factors contributing to the variable response of clopidogrel, there are non-genetic factors which contribute as well. Diabetes mellitus (DM), chronic kidney disease (CKD), smoking, age, obesity, diet, fast platelets turn over, increased exposure to ADP stimulation and drug-drug interactions were found to interfere with clopidogrel's response (Shuldiner et al., 2009; Angiolillo and Luis Ferreiro, 2010; Bouman et al., 2011; Yin and Miyata, 2011; Su et al., 2012; Ferri et al., 2013; Gremmel et al., 2013; Lewis, Horenstein, et al., 2013; Scott et al., 2013).

The presence of comorbid condition such as type 2 DM increases platelets reactivity in CAD patients which may cause poor outcome (Angiolillo et al., 2005; Angiolillo et al., 2011). In fact, type 2 DM patients were found to have increased in hyporesponsiveness to clopidogrel treatment and increased platelets reactivity while on DAPT (Angiolillo et al., 2005). Furthermore, type 2 DM patients had reduced response to high loading dose of clopidogrel (600 mg) (Geisler et al., 2007). Notably, there is an inter individual clopidogrel's response variability among CAD patients with